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(54) Immunostimulatory remedies containing palindromic DNA sequences.

(57) Single-stranded, linear polydeoxyribonucleotides with a base number of 10 to 100 containing at least one structure represented by the following general formula:

$$5'\text{-}X_n \cdots X_3 X_2 X_1 Y_1 Y_2 Y_3 \cdots Y_n\text{-}3' \quad (I)$$

(wherein n is an integer from 3 to 50; X_1, X_2, X_3, $\cdots$, X_n and Y_1, Y_2, Y_3, $\cdots$, Y_n are each a monodeoxyribonucleotide; X_1, X_2, X_3, $\cdots$ and X_n may be the same or different nucleotides; and bases in X_1 and Y_1, in X_2 and Y_2, in X_3 and Y_3, in $\cdots$, and in X_n and Y_n are complementary with each other as defined by Watson & Crick), and double-stranded, linear polydeoxyribonucleotides, in which at least one single-stranded, linear polydeoxyribonucleotide contains at least one structure represented by the general formula (I), both show strong immunostimulatory activity.

Thus, remedies containing, as active ingredient, such a specific polydeoxyribonuleotide as dsecribed above, or a salt thereof, are efficacious against malignant tumors, infectious diseases, immunodeficiency diseases and autoimmune diseases, with minimized side-effects.

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Thus, remedies containing, as active ingredient, such a specific polydeoxyribonuleotide as dsecribed above, or a salt thereof, are efficacious against malignant tumors, infectious diseases, immunodeficiency diseases and autoimmune diseases, with minimized side-effects.

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Th structure represented by the general formula (I) of this invention is a sequence of monodeoxyribonucleotides called palindromic structure, in which X_1 and Y_1 , X_2 and Y_2, X_3 and Y_3, ••• , and X_n and Y_n are complementary with each other as defined by Watson & Crick. A palindromic structure generally means on of the symmetric structures found in a double-stranded DNA, and these structures are the recognition site for many kinds of restriction enzymes. In this invention, this structural nomenclature commonly employed for double-stranded DNAs is used also for single-stranded DNAs, for convenience, and the structure represented by the general formula (I) is hereinafter called the palindromic structure or palindromes.

What is to be noticed here is that the single- or double-stranded DNAs which are entirely composed of alternately repeated sequence of only two types of monodeoxynucleotides (for example, G and C, or A and T) cannot achieve the purpose of this invention. "n" in the general formula (I) is an integer from 3 to 50.

The following sequences are examples of the desirable structure of the present invention,wherein G is a deoxyguanylic acid, A is a deoxyadenylic acid, C is a deoxycytidylic acid and T is a deoxythymidylic acid, and wherein the left side is 5'-terminal and the right side is 3'-terminal in each sequence:

GAGCTC, TAGCTA, AGGCCT, CATATG, TGTACA, GTTAAC, GATATC,

GGGCCC, TTGCAA (n = 3)

GTAGCTAC, AAGGCCTT, GGATATCC, CAGGCCTG, GCATATGC, GTGTACAC,

AGTTAACT (n = 4)

AGTAGCTACT, GAAGGCCTTC, AGGATATCCT, GCAGGCCTGC,

AGCATATGCT (n = 5) ••••••

To achieve the purpose of this invention, it is more desirable that one or more of the 5'-CG-3' structure be included in the structure represented by the general formula (I). As examples of such a structure, may be mentioned the following sequences:

CGATCG, ATCGAT, TCGCGA, AACGTT, GCGCGC, CGTACG, AGCGCT,

CGGCCG, GACGTC, GTCGAC, CGCGCG, ACGCGT, CACGTG (n = 3)

ACGATCGT, GATCGATC, ATCGCGAT, CAACGTTG, AGCGCGCT, ACGTACGT,

TAGCGCTA, ACGGCCGT, CGACGTCG, CGTCGACG (n = 4)

GACGATCGTC, CGATCGATCG, GATCGCGATC, GCAACGTTGC,

CAGCGCGCTG, GACGTACGTC, CTAGCGCTAG, GACGGCCGTC, ACGACGTCGT,

ACGTCGACGT, ACAACGTTGT (n = 5) •••••••

The single-stranded, linear DNA of this invention is an unbranched DNA molecule in which each of the compon nt monodeoxyribonucl otides is linked to the adjacent monodeoxyribonycleotide through a[5'-3'] phosphodiest r bond. Th single-stranded, lin ar DNAs carrying less than six bases, which fail to satisfy the general formula (I), are not satisfactory. As may b apparent from the Examples described later, the longer the chain length of single-stranded, linear DNA, the better will be the result. However, the purpose of this invention may b sufficiently achieved with a DNA with a bas number in the range from 10 to 100, because

functions of immune system are suppressed or lost, such as agammaglobulinemia and acquired immunodeficiency syndromes. Among the patients of these diseases, the morbidity of infectious diseases and malignant tumors is high, thus adversely affecting recuperation. DNAs of this invention, which are efficacious against malignant tumors and are also capable of inducing interferon, are expected to encourage the recuperation of the patients suffering immunodeficiency diseases by curing the malignant tumors and infectious diseases which are likely to concur in these patients.

Single- and double-stranded, linear DNAs of this invention may be administerd to animal and human bodies subcutaneously, intravenously, intramuscularly, intratumorally, orally or into the rectum, and the suitable administration route should be selected case by case depending on the type of disease and the conditions of the patient. For example, intratumoral or subcutaneous administration is preferable in the case of malignant tumors. The proper dose to humans is 1 to 1000 mg/day when administered into the rectum or orally, and 0.01 to 100 mg/day when administered subcutaneously, intravenously, intratumorally or intramuscularly. Administration should be repeated once or twice per one to seven days, preferably once per one or two days, and the frequency of administration may be varied and the period of administration may be further prolonged, as required.

When administering single- or double-stranded, linear DNAs of this invention to animal and human bodies subcutaneously, intravenously, intramuscularly or intratumorally, it is preferable to appply it in the form of an injection prepared by dissolving the DNA in an aqueous solution which is nearly neutral (pH 5 to 8) with a physiological osmotic pressure. As examples of such an aqueous solution, may be mentioned the isotonic sodium chloride solution specified in Pharmacopoeia of Japan, and aqueous solutions containing salts, compounds, additives or diluents medicinally approved. The single- and double-stranded, linear DNAs of this invention may be used as an injection either in the form of an aqueous solution as described above or in the form of solid obtained by lyophylizing the same.

The single- and double-stranded, linear DNAs of this invention, when orally administered to animal and human bodies, may be used in the form of capsules, granules, pills, fine granules, tablets or syrup, as in the case of common drugs.

The fact that a specific base sequence in a DNA molecule has an important effect upon its immunostimulatory activity has not been known at all, and this is a completely new finding.

DNAs of this invention enhance the production of interferon and macrophage activating factor, thus activating NK cells and macrophages, also enhance the production of colony-stimulating factor, promote the proliferation of lymphocytes, and are therefore considered to exhibit a wide range of immunostimulatory activity. In addition, these DNAs proved to be very efficacious remedies against experimental tumors, and experimental models for immunodeficiency diseases and for autoimmune diseases, through their immunostimulatory activity. Furthermore, the acute toxicity of these DNAs is much lower than that of synthetic RNA; thus these DNAs are expected to be highly efficacious and useful remedies against malignant tumors, various auto-immune diseases, immunodeficiency diseases and infectious diseases.

EXAMPLES

Detailed below are Test Methods and Examples, in which guanine, adenine, cytosine and thymine (bases contained in nucleic acids) are abbreviated as G, A, C and T, respectively, and the nucleotide sequence in each DNA molecule is represented so that the left side is 5'-terminal and the right side is 3'-terminal. The base sequence in each DNA used in the Examples is shown in the Sequence List appended at the end of this specification. The complex of polyinosinic acid and polycytidylic acid (hereinafter abbreviated as poly I:C), which is a synthetic RNA used as the control remedy, was purchased from Yamasa Shoyu.

Test Method 1 (In-vitro tests on the augmentation of mouse NK-cell activity, on the production of interferon, and on the production of macrophage activating factor)

The tests were performed according to the known method described in the following literature:

Yamamoto, S., et al.; Jpn. J. Cancer Res., 79, 866-873 (1988)

provided that the NK-cell activity was measured by a four-hour ^{51}Cr release assay using YAC-1 cells as targets, and the result was expressed by the average of triplicate measurements and the standard deviation. The effector to target ratio was 100:1 unless otherwise stated.

Test Method 2 (Titration of colony-stimulating factor)

A solution of DNA in PBS was administered to MRL·MPJ-lpr mice (female, six weeks old) subcutaneously three times per week, and the amount of protein in the urine was calculated from the urine volume excreted during l6 hours and the protein concentration therein.

Test Method 7 (Acute toxicity test in mice)

DNA or RNA was administered to ICR mice (l0 mice per group) intravenously (iv) or intraperitoneally (ip), and the number of living mice was counted 24 hours after administration. Graphs were made in which the ratio of living mice was plotted against the amount of DNA or RNA administered, and the amount of DNA or RNA per body weight that will kill half of the mice was estimated - LD_{50}) mg/kg).

Test Method 8 (Test of efficacy against mice infected with LP-BM5 viruses)

Stock solution of LP-BM5 viruses (0.5 ml) was injected intraperitoneally to each of C57BL/l0 mice (five weeks old). From the next day, mice were fed with drinking water ad libitum to which azidothymidine (AZT) used as a control drug, was added at a concentration of 0.5 mg/ml. DNA was administered intraperitoneally every day in an amount of l mg as a solution in PBS. Five weeks after the virus infection, the spleen cells obtained from the mice were suspended in RPMIl640 medium containing l0% FCS at a cell concentration of l x $l0^7$/ml. After culturing the cells at 37° C for 20 hours under 5% CO_2 in the presence of IL-2 (l000 U/ml, a product of Genzyme Corp.), NK cell activity was measured.

Test Method 9 (Quantitation of DNA)

DNA was dissolved in 0.2mM phosphate buffer (pH 7.0), and the absorbance at 260 nm was measured with the same buffer used as reference. DNA concentration was determined by assuming that the concentration of DNA that gave an absorbance of 1 was 20 µg/ml.

Example 1 (Preparation of tablets containing, as active ingredient, a single-stranded, linear DNA of this invention)

A mixture of 5 g of sodium salt of a single-stranded, linear DNA (Sequence 1), 53 g of lactose, 50 g of corn starch and 35 g of crystalline cellulose was kneaded with a solution of 5 g hydroxypropylcellulose in l0 ml water to form granules, which were dried at 50°C for four hours. Magnesium stearate (2 g) was then admixed, and the mixture was compressed into tablets (each weighing 200 mg) by the use of a tabletting machine.

Example 2 (Preparation of capsules containing, as active ingredient, a single-stranded, linear DNA of this invention)

A mixture of 5 g of sodium salt of a single-stranded, linear DNA (Sequence 1), 124 g of lactose, 90 g of corn starch, 70 g of crystalline cellulose and 11 g of magnesium stearate was filled into hard gelatin capsules (300 mg in each) by the use of a capsule filling machine, thus giving capsules.

Example 3 (Preparation of parenteral injections containing, as active ingredient, a single-stranded, linear DNA of this invention)

One gram of sodium salt of a single-stranded, linear DNA (Sequence 1) and 0.5 g of sodium chloride were dissolved in 1 liter of distilled water for injection, and the mixture was filtered and sterilized, thus giving injections.

Example 4

The fact that DNAs containing palindromic structure have stronger immunopharmacological activity than those containing no palindromic structur was demonstrated by th following exp riments.

A single-stranded, linear DNA (Sequ nc 1) with a base number of 45 containing a palindromic structure (GACGTC) had a strong activity to augment the mouse NK-cell activity (Table 1). In contrast, a single-strand d, lin ar DNA (Sequence 2) with a base number of 45 containing no palindromic structure had a weak r activity to augment th mouse NK-cell activity (Tabl 1).

palindromic structure was replaced with a reversed sequence of nucleotides (TG), had a strong activity to augment the NK-cell activity like the DNA (Sequence 3) (Table 3).

The DNA (Sequence 7), which is of much the same structure as the DNA (Sequence 3) except that one nucleotide (C) in the palindromic structure is lacking, had a markedly weaker activity to augment the NK-cell activity than the DNA (Sequence 3) (Table 3). However, the DNA (Sequence 8), which is of much the same structure as the DNA (Sequence 3) except that one nucleotide (C) in the portion other than the palindromic structure is lacking had a strong activity to augment the NK-cell activity like the DNA (Sequence 3) (Table 3).

The DNA (Sequence 9), which is of much the same structure as the DNA (Sequence 3) except that the palindromic structure is translocated to the 5'-terminal, had as strong an activity to augment the NK-cell activity as that of the DNA (Sequence 3) (Table 3); however, the DNA (Sequence 10), in which the palindromic structure is translocated to the central position, had a stronger activity to augment the NK-cell activity than that of the DNA (Sequence 3) (Table 4).

Table 3

Test Sample	Number of Bases	NK-Cell Activity
Control		14.1±2.0
Sequence 3	30	48.8±2.6
Sequence 5	30	15.8±1.5
Sequence 6	30	45.3±2.3
Sequence 7	29	15.4±1.4
Sequence 8	29	50.9±1.9

Each DNA was added to spleen cells to a final concentration of 50 μg/ml.

Table 4

Test Sample	Number of Bases	NK-Cell Activity
Control		14.2±0.9
Sequence 3	30	43.4±2.1
Sequence 9	30	45.8±2.6
Sequence 10	30	51.4±2.8

Each DNA was added to spleen cells to a final concentration of 50 μg/ml.

It was demonstrated from the above results that the presence of the palindromic structure (GACGTC) has an important effect upon the DNA's activity to augment the NK-cell activity, and that a higher activity can be obtained if the palindromic structure is located in the central position of the DNA chain.

Example 5

Example 6

The following experiment demonstrated that DNAs containing a palindromic structure with a base number of 8 or 10 also have a high immunopharmacological activity. The DNA (Sequence 21) containing a palindromic structure (GACGTC), and the DNAs (Sequences 22 and 23) containing an expanded palindromic structure (GGACGTCC, CGGACGTCCG), had a strong activity to augment the NK-cell activity (Table 7). However, the DNA (Sequence 24) containing a curtailed GACGTC structure (ACGT) had only a low activity.

Table 7

Test Sample	Palindrome	NK-Cell Activity
Control		14.9±1.0
Sequence 24	ACGT	15.0±0.3
Sequence 21	GACGTC	42.3±1.1
Sequence 22	GGACGTCC	45.5±2.2
Sequence 23	CGGACGTCCG	52.6±1.5

Each DNA was added to spleen cells to a final concentration of 50 μg/ml.

Example 7

The fact that the immunopharmacological activity of DNAs containing a palindromic structure depends on the molecular length was discovered from the results of the following experiments.

Comparison of the activity to augment the NK-cell activity among DNAs (Sequences 25 through 32) with a palindromic structure in the central position and having a variety of molecular lengths (number of bases: 6 to 80) showed that the activity was observed only in the DNAs having 10 or more bases, increased with the number of bases, and changed little when the number of bases exceeded 45 (Table 8).

activity to augment the NK-cell activity (Table 10).

Table 10

Test Sample	Palindrome	NK-Cell Activity
Control		14.2±0.5
Sequence 37	GACGTC	45.1±3.2
Sequence 38	CACGTG	47.6±2.9
Sequence 39	AACGTT	42.5±3.2

Each DNA was added to spleen cells to a final concentration of 20 μg/ml.

Example 9

The palindrome-containing DNA which carries only guanine and cytosine as component base (Sequence 40) and the one which carries only adenine and thymine as component base (Sequence 41) had only a weak activity to augment the NK-cell activity (Table 11).

Table 11

Test Sample	Palindrome	Component Base	NK-Cell Activity
Control			14.4±1.6
Sequence 3	GACGTC	G, A, C, T	46.3±2.5
Sequence 40	GCGCGC	G, C	14.9±1.3
Sequence 41	ATATAT	A, T	14.5±1.4

Each DNA was added to spleen cells to a final concentration of 50 μg/ml.

It was demonstrated from the above results that, in order for a DNA to exhibit a satisfactory immunopharmacological activity, it must contain at least one palindrome composed of six or more bases; the total number of bases contained therein must be ten or more; and the sequences entirely composed of repetition of GC- or AT-array are unfavorable.

Example 10

Single-stranded, linear DNAs containing palindromic structure induced interferon (hereinafter abbreviated as IFN) and macrophage activating factor (hereinafter abbreviated as MAF).

Th DNA (Sequence 1) containing a palindromic structure with a base number of 45 had an in-vitro activity to induce IFN and MAF from mouse spleen cells, but the activity of the DNA (Sequence 2) without palindromic structure was weaker (Tabl 12).

Example 12

ConA-stimulated proliferation of spleen cells was promoted in mice to which 5 mg of the palindrome-containing DNA (Sequence 1) with a base number of 45 had been administered (Table 14). However, no such activity was observed with the DNA (Sequence 2) without palindromic structure.

Table 14

Test Sample	Days after Administration	S.I.
Control		42.3
Sequence 1	1	76.1**
	2	66.7*
	3	47.9*
Sequence 2	1	45.5
	2	43.1
	3	42.0

*: $p<0.05$, **: $p<0.01$ (by Student's t-test)

The above results demonstrated that DNAs containing palindromic structure exhibit a variety of immnopharmacological activities.

Example 13

When the palindrome-containing DNA (Sequence 1) with a base number of 45 was administered to mice bearing IMC carcinoma tumors, dose-dependent suppression of the tumor weight (namely, antitumor activity) was observed (Table 15). However, the DNA (Sequence 2) with a base number of 45 without palindromic structure had only a weak antitumor activity (Table 15).

Example 15

Administration of the single-stranded, linear DNA (Sequence 1) containing a palindromic structure to the autoimmune disease model, MRL MPJ-lpr mice which suffer spontaneous outbreak of such diseases, suppressed the amount of protein excreted in the urine (Table 17). However, no such activity was observed with the DNA (Sequence 2) without palindromic structure.

Table 17

Test Sample	Dose (mg/kg)	Protein Content in the Urine (mg)	
		At the Start	After 4 Weeks
Control	0	0.7±0.5	4.0±2.6
Sequence 1	0.03	0.7±0.5	3.1±1.8
	0.3	0.7±0.5	3.3±1.S
	3	0.6±0.4	3.5±1.3
Sequence 2	0.03	0.7±0.6	4.1±2.3
	0.3	0.5±0.4	3.5±1.6
	3	0.7±0.5	3.9±3.0

Table 17 (contd.)

Test Sample	Dose (mg/kg)	Protein Content in the Urine (mg)	
		After 8 Weeks	After 12 Weeks
Control	0	7.1±13.5	13.6±22.2
Sequence 1	0.03	2.5±3.1	4.5±6.1
	0.3	1.7±1.1*	8.6±12.3
	3	3.2±5.8	9.7±16.1
Sequence 2	0.03	7.3±9.5	12.6±6.2
	0.3	5.8±5.2	10.7±12.3
	3	5.4±4.0	13.0±18.8

*: $p<0.05$ (Student's t-tgest)

The above results demonstrated that synthetic DNAs containing palindromic structure have not only immunopharmacological activities but also therapeutic effects on various diseases which are known to be susceptible to drugs with immunopharmacological activity.

Table 20

Test Sample	Palindrome	NK-Cell Activity
Control		12.3±0.6
Sequence 11	AACGTT	50.0±1.8
Sequence 12	AGCGCT	48.2±1.9
Sequence 49	CGATCG	47.7±1.4
Sequence 50	ATCGAT	47.0±1.6
Sequence 51	TCGCGA	47.0±2.0
Sequence 52	GCGCGC	46.9±1.2
Sequence 53	CGTACG	46.6±1.9
Sequence 54	AGCGCT	45.7±1.0
Sequence 55	CGGCCG	44.2±1.7
Sequence 3	GACGTC	42.1±1.5
Sequence 56	GTCGAC	42.0±1.3
Sequence 57	CGCGCG	40.1±1.4
Sequence 58	ACGCGT	39.5±1.5
Sequence 59	AAGCTT	20.1±0.8
Sequence 60	TTATAA	19.9±0.5
Sequence 61	TATATA	18.8±0.4
Sequence 62	AGTACT	18.0±0.7
Sequence 63	GAATTC	17.7±0.6
Sequence 64	CTGCAG	17.5±0.5
Sequence 65	AAATTT	17.5±0.7
Sequence 66	CCTAGG	17.0±0.7

Each DNA was added to spleen cells to a final concentration of 50 μg/ml.

Example 19

In mice infected with LP-BM5 viruses (model animals for immunodeficiency diseases), the NK-cell activity of th lymphocytes was not enhanced by IL-2 stimulation unlike in normal mice; however, in the virus-infected mice to which th single-stranded, linear DNA (Sequ nce 1) containing a palindromic structure had been administered, IL-2 was able to enhance the NK-cell activity of the lymphocytes (Table 21). The degree of enhancement was nearly th same as that observed in the IL-2-stimulated lymphocytes

Table 22

Mouse	Test Sample	NK-Cell Activity	IFN Titer (U/ml)
BALB/c	Control	5.2±0.5	<4
	Sequence 1	15.4±0.7	120
	Sequence 2	5.4±0.3	<4
SCID	Control	18.3±1.0	16
	Sequence 1	43.9±1.5	256
	Sequence 2	20.0±1.1	16

Each DNA was added to spleen cells to a final concentration of 20 μg/ml.

NK-cell activity was measured at an E:T ratio of 25:1.

It was demonstrated from the results obtained in Examples 19 and 20 that the single-stranded, linear DNAs of this invention containing palindromic structure are capable of restoring (at least partially) the immunological functions of immunodeficiency disease model mice.

Example 21

Acute toxicity of the synthetic DNAs (Sequences 1 and 3) with a base number of 45 and 30, respectively, was remarkably low as compared to that of the synthetic RNA (polyI:C) used as control (Table 23).

Table 23

Test Sample	Administration Route	LD_{50} (mg/kg)
DNA (Sequence 1)	iv	>500
	ip	>1000
DNA (Sequence 3)	iv	>500
	ip	>1000
polyI:C	iv	8
	ip	30

In addition, intraperitoneal administration of each of the synthetic DNAs (Sequences 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 42, 43, 44, 45, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58) to DDY mice (each group consisting of ten heads), caused no death or body weight loss in any of the mice tested in one-week observation period.

Sequence No.: 4

Length of sequence: 30

Type of sequence: Nucleic acid:

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence:

GGTGACGGCA CCACGACGGC CACCGTGCTG

Sequence No.: 5

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence:

ACCGATGACT GCGCCGGTGA CGGCACCACG

Sequence No.: 6

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

ACCGATGACG TCGCCGTGGA CGGCACCACG

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Sequence No.: 10

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

ACCACGACCG ATGACGTCGC CGGTGACGGC

Sequence No.: 11

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (AACGTT)

ACCGATAACG TTGCCGGTGA CGGCACCACG

Sequence No.: 12

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (AGCGCT)

ACCGATAGCG CTGCCGGTGA CGGCACCACG

Sequence No.: 16

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topologty: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (ATCGAT)

TCGGTGATCG ATATGTCGCA GGACCCGGTC

Sequence No.: 17

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topologty: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (TCGCGA)

TCGGTGTCGC GAATGTCGCA GGACCCGGTC

Sequence No.: 18

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GCGCGC)

AAAAGAAGTG GGGACGTCTT ACGATCACCA

Sequence No.: 22

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GGACGTCC)

AAAAGAAGTG GGGACGTCCT ACGATCACCA

Sequence No.: 23

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (CGGACGTCCG)

AAAAGAAGTG CGGACGTCCG ACGATCACCA

Sequence No.: 24

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (ACGT)

CCGATGACGT CGCCG

Sequence No.: 28

Length of sequence: 20

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

GACCGATGAC GTCGCCGGTG

Sequence No.: 29

Length of sequence: 30

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

TGACAGACCG ATGACGTCGC CGGTGGACGG

Sequence No.: 30

Length of sequence: 45

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequen e: P: Contains palindrom (GACGTC)

Features of sequence: P: Contains palindrome (GACGTC)

ACCGATGACG TCGCGACGTC CGGCACCACG ACGGCCACCG TGCTG

Sequence No.: 34

Length of sequence: 45

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

ACCGATGACG TCGCGACGTC CGGACGTCCG ACGGCCACCG TGCTG

Sequence No.: 35

Length of sequence: 45

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

ACCGATGACG TCGCGACGTC CGGACGTCCG GACGTCACCG TGCTG

Sequence No.: 36

Length of sequence: 45

Type of sequence: Nucleic acid

Type of Chain : Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (AACGTT)

AACGTTAACG TTAACGTTAA CGTTAACGTT

Sequence No.: 40

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GCGCGC)

GCGCGCGCGC GCGCGCGCGC GCGCGCGCGC

Sequence No.: 41

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (ATATAT)

ATATATATAT ATATATATAT ATATATATAT

Sequence No.: 42

Length of sequence: 45

Type of sequence: Nucleic acid

Type of chain: Double-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

TTTTTTTTTT TTGACGTCTT TTTTTTTTTT

Sequence No.: 46

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GACGTC)

CCCCCCCCCC CCGACGTCCC CCCCCCCCCC

Sequence No.: 47

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome(GACGTC)

GCGCGCGCGC GCGACGTCGC GCGCGCGCGC

Sequence No.: 48

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (TCGCGA)

ACCGATTCGC GAGCCGGTGA CGGCACCACG

Sequence No.: 52

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GCGCGC)

ACCGATGCGC GCGCCGGTGA CGGCACCACG

Sequence No.: 53

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (CGTACG)

ACCGATCGTA CGGCCGGTGA CGGCACCACG

Sequence No.: 54

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (TCGCGA)

ACCGATTCGC GAGCCGGTGA CGGCACCACG

Sequence No.: 52

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (GCGCGC)

ACCGATGCGC GCGCCGGTGA CGGCACCACG

Sequence No.: 53

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (CGTACG)

ACCGATCGTA CGGCCGGTGA CGGCACCACG

Sequence No.: 54

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (CGCGCG)

ACCGATCGCG CGGCCGGTGA CGGCACCACG

Sequence No.: 58

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (ACGCGT)

ACCGATACGC GTGCCGGTGA CGGCACCACG

Sequence No.: 59

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (AAGCTT)

ACCGATAAGC TTGCCGGTGA CGGCACCACG

Sequence No.: 60

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Top logy: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrom (GAATTC)

ACCGATGAAT TCGCCGGTGA CGGCACCACG

Sequence No.: 64

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (CTGCAG)

ACCGATCTGC AGGCCGGTGA CGGCACCACG

Sequence No.: 65

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucleic acid; synthetic DNA

Features of sequence: P: Contains palindrome (AAATTT)

ACCGATAAAT TTGCCGGTGA CGGCACCACG

Sequence No.: 66

Length of sequence: 30

Type of sequence: Nucleic acid

Type of chain: Single-stranded

Topology: Linear

Kind of sequence: Other nucl ic acid; synthetic DNA

general formula (I) is 5'-TCGCGA-3' (wherein G is deoxyguanylic acid, A is deoxyadenylic acid, C is deoxycytidylic acid, and T is deoxythymidylic acid).

12. An immunostimulatory remedy as described in Claim 1 or 2, wherein the structure represented by the general formula (I) is 5'-AACGTT-3' (wherein G is deoxyguanylic acid, A is deoxyadenylic acid, C is deoxycytidylic acid, and T is deoxythymidylic acid).

13. An immunostimulatory remedy as described in Claim 1 or 2 , wherein the structure represented by the general formula (I) is 5'-GCGCGC-3' (wherein G is deoxyguanylic acid, and C is deoxycytidylic acid).

14. An immunostimulatory remedy as described in Claim 1 or 2, wherein the structure represented by the general formula (I) is 5'-CGTACG-3' (wherein G is deoxyguanylic acid, A is deoxyadenylic acid, C is deoxycytidylic acid, and T is deoxythymidylic acid).

15. An immunostimulatory remedy as described in Claim 1 or 2 , wherein the structure represented by the general formula (I) is 5'-CGGCCG-3' (wherein G is deoxyguanylic acid, and C is deoxycytidylic acid).

16. An immunostimulatory remedy as described in Claim 1 or 2 , wherein the structure represented by the general formula (I) is 5'-GTCGAC-3' (wherein G is deoxyguanylic acid, A is deoxyadenylic acid, C is deoxycytidylic acid, and T is deoxythymidylic acid).

17. An immunostimulatory remedy as described in Claim 1 or 2 , wherein the structure represented by the general formula (I) is 5'-CGCGCG-3' (wherein G is deoxyguanylic acid, and C is deoxycytidylic acid).

18. An immunostimulatory remedy as described in any of Claims 1 through 17 , wherein the portion except the structure represented by the general formula (I) is the repeated structure of deoxyguanylic acid.

19. An immunostimulatocy remedy as described in Claim 1 or 2 , wherein the structure represented by the general formula (I) contains at least one structure of 5'-CG-3' (wherein G is deoxyguanylic acid, and C is deoxycytidylic acid).

20. An immunostimulatory remedy as described in Claim 19 , wherein the portion except the structure represented by the generagl formula (I) is the repeated structure of deoxyguanylic acid.

21. An immunostimulatory remedy as described in any one of Claims 1 to 20, for the relief of malignant tumors, infectious dieases, immunodeficiency diseases and autoimmune diseases.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 11 2601

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 300 687 (CITY OF HOPE) * page 2, line 48 - line 49 * ---	1-2	A61K31/70
X	EP-A-0 302 758 (NEW ENGLAND MEDICAL CENTER HOSPITALS) * claims 2-4 * ---	1-2	
X	DE-A-3 744 785 (THEURER K.) * the whole document * ---	1-2	
A	WORLD PATENTS INDEX LATEST Week 9026, Derwent Publications Ltd., London, GB; AN 90-196689 & JP-A-2 128 691 (NIPPON OIL SEAL) 17 May 1990 * abstract * ---	1-21	
A,P	MEDLINE ABSTRACT Number: 90338013 WEGNER M. et al:"Interaction of a protein with a palindromic sequence from murine RDNA increases the occurrence of amplification-dependent transformation in mouse cells." & J.BIOL.CHEM. 15 august 1990, 265 (23), p 13925-32 -----	1-21	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	22 APRIL 1992	AVEDIKIAN P.F.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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